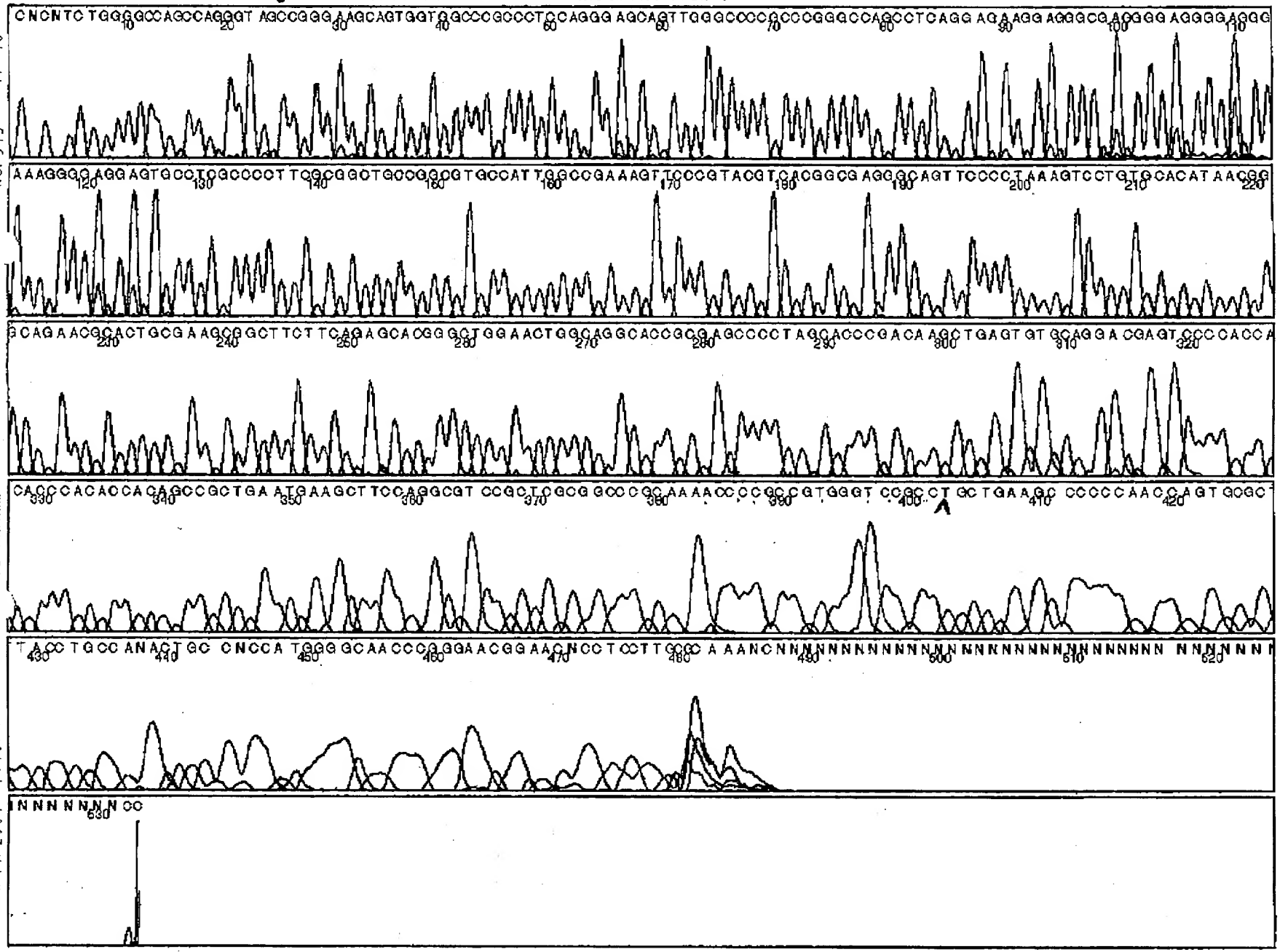
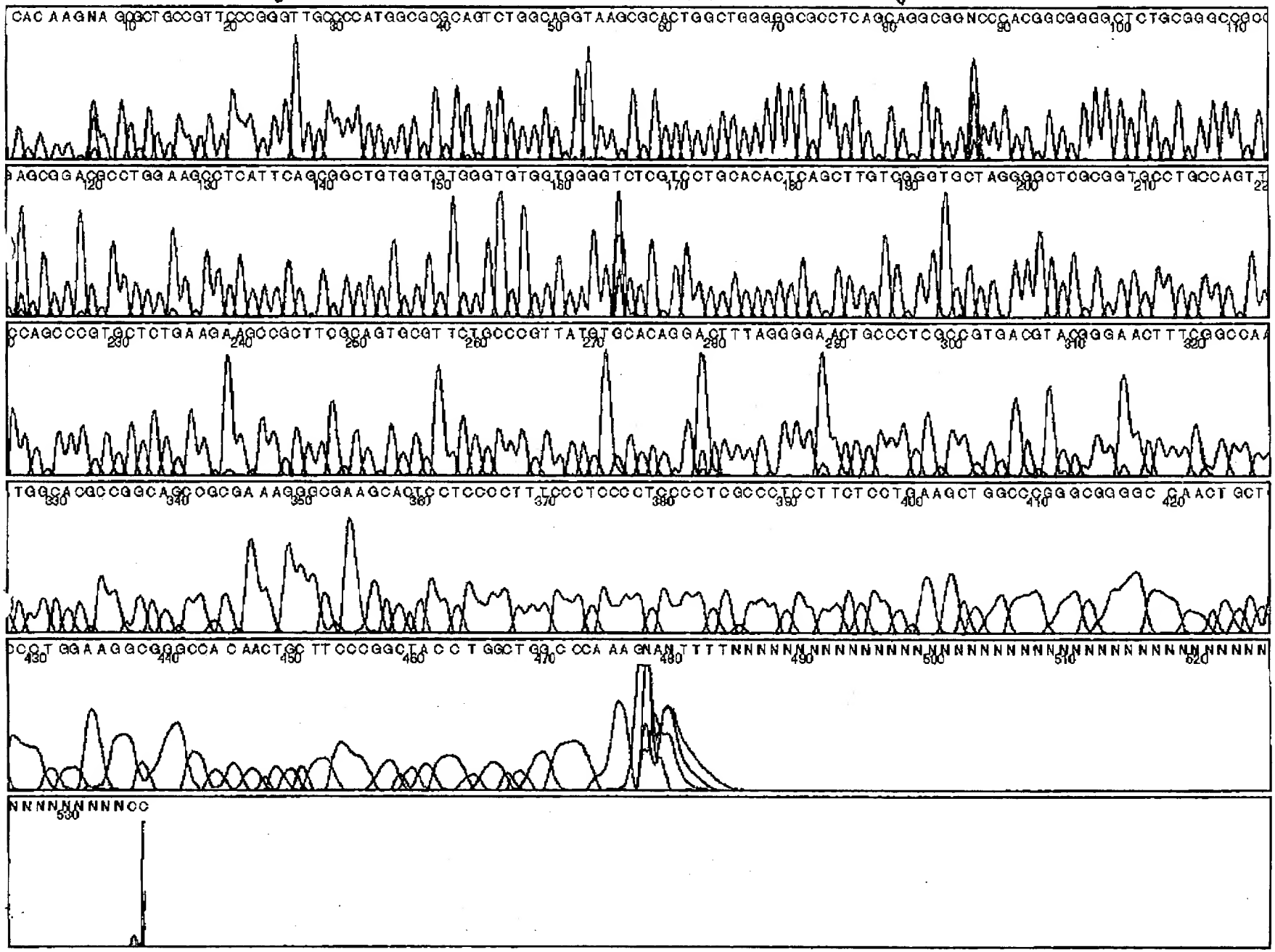
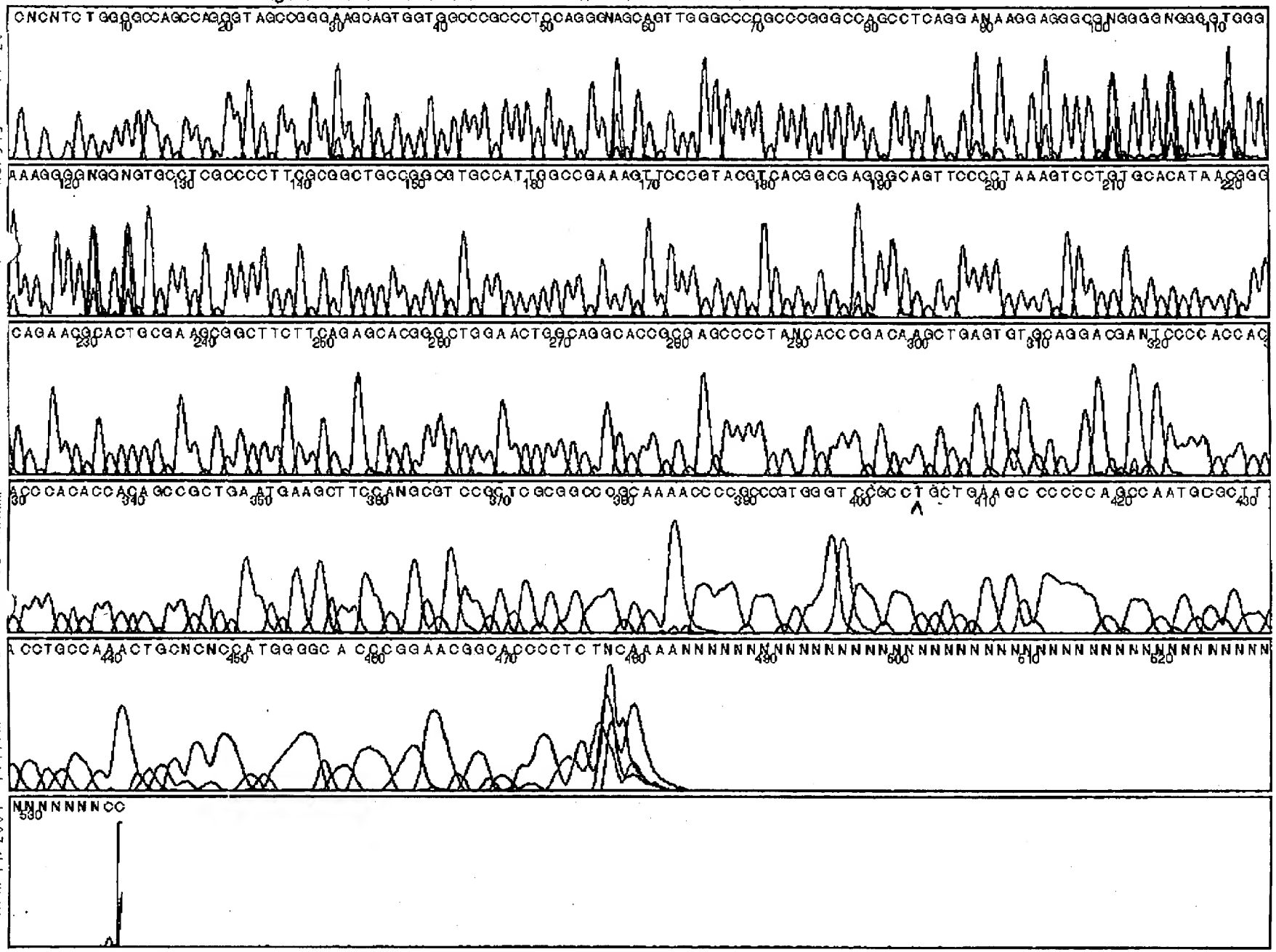
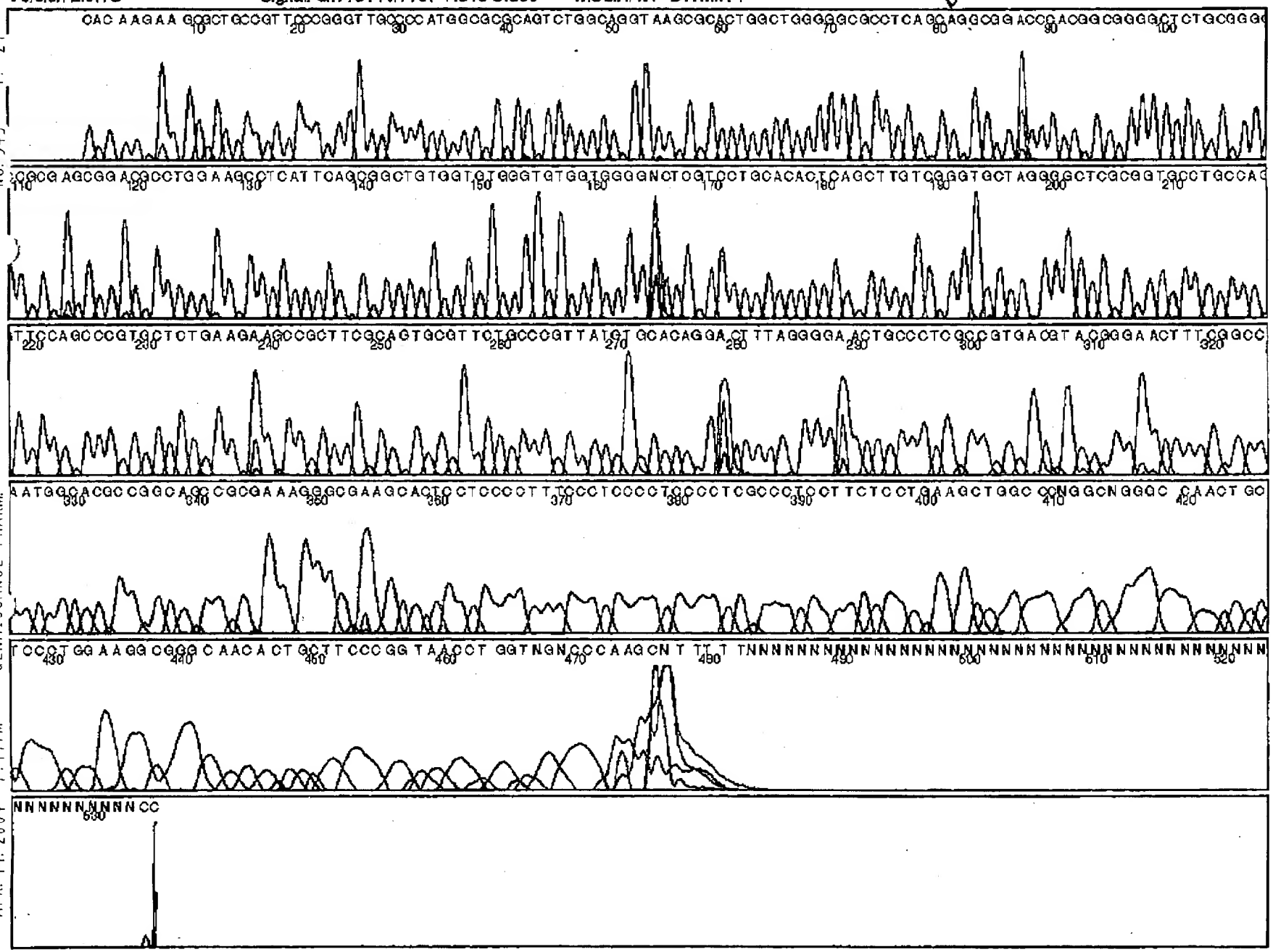
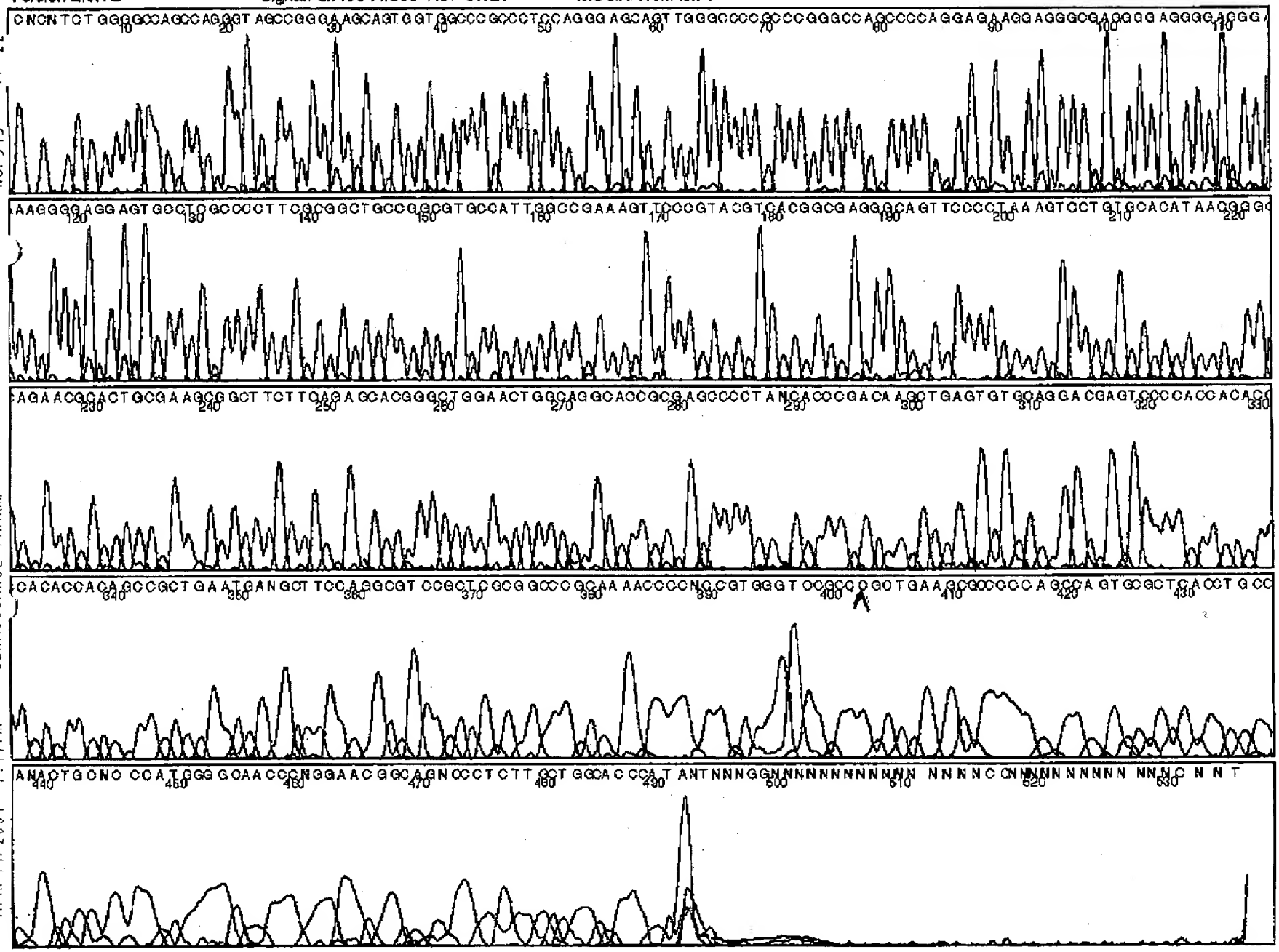
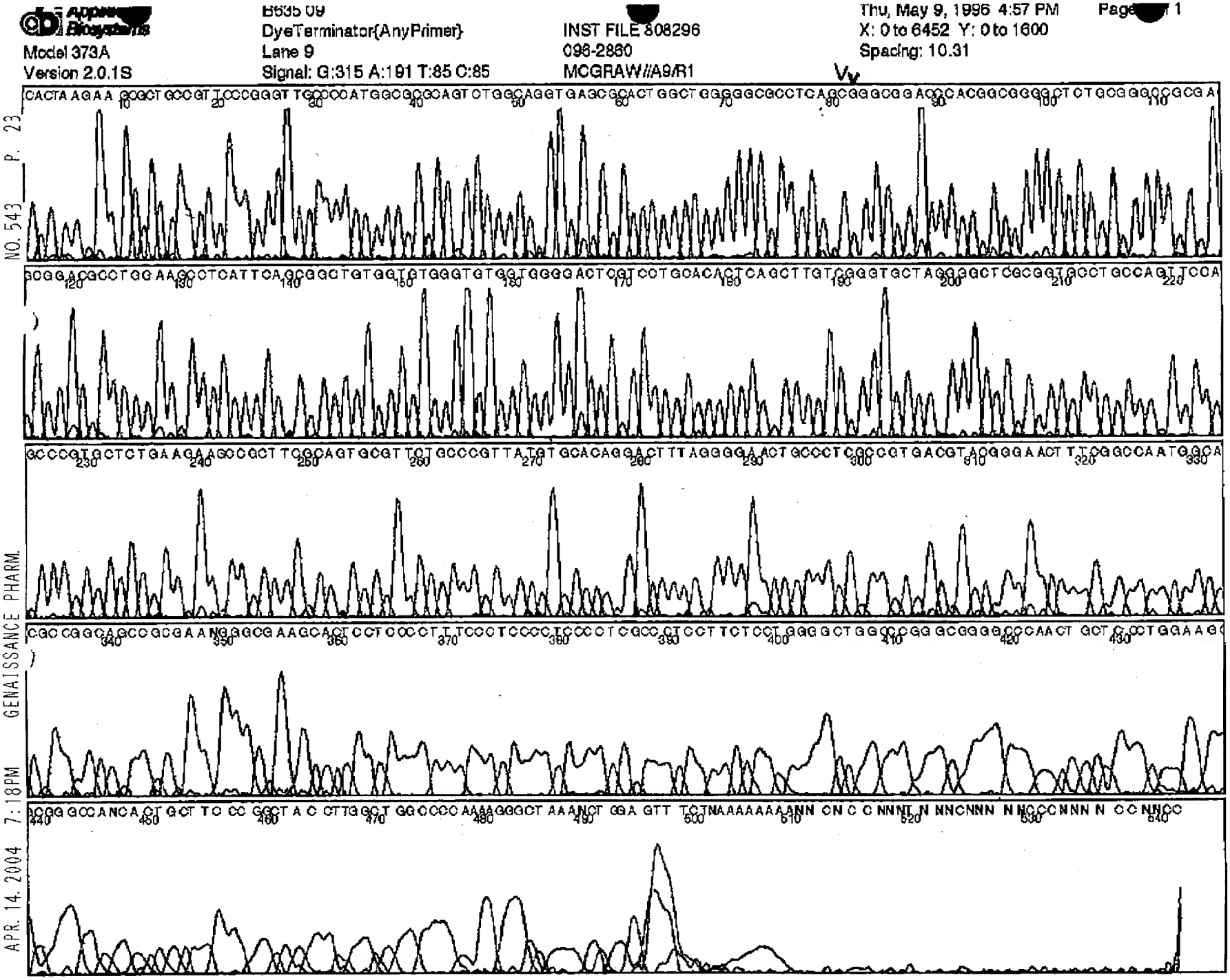












PCR (β_3AR) 1/26/96

* Made new set of primers for β_3AR 5' flanking region. Product should include the CRE + short ORF. Primers were chosen ± MacVector

Blood samples are being obtained from pts in the UC asthma clinic by Melanie Meyers.

DNA samples prepared by Liz Donnelly using Invitrogen +/- Qiagen kits. Samples are numbered as received (A1, A2, etc.)

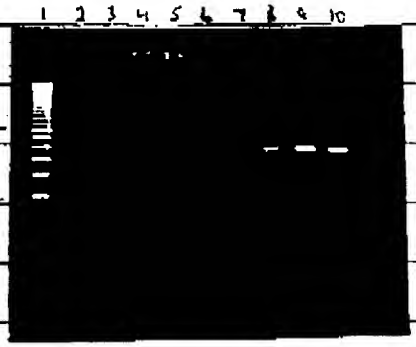
- Set up PCR rxn using new primers. Made up 96 well master mix:
  - 10 µl buffer II
  - 6 µl 25mM $MgCl_2$
  - 0.8 µl 25mM dNTP
  - 0.5 µl 100µM forward primer (β_3AR-F1)
  - 0.5 µl 100µM reverse primer (β_3AR-R1)
  - 77.7 µl dH_2O
  - 0.5 µl AmpliTaq
- dispense 94 µl of master mix into PCR tube
- add 1µl template DNA
- overlay ± 1 drop mineral oil
- perform PCR in Stratagene robocycler ± temp gradient mode on
  - 94°C x 2 min
  - 94°C x 30 sec / 64°, 63°, 60°, or 58°C x 30 sec / 72°C x 30 sec => 35 cycles
  - 72°C x 7 min
- run 10µl of rxn on TBE minigel

1/26/96 (cont)

see GAK RIA

region 1

original plan (1/26/96)



1 - 100 bp ladder		6 - blank	
2 - A1 (Invitrogen)	64°C	7 - A1(Qiagen)	64°C
3 - "	62°C	8 - "	62°C
4 - "	60°C	9 - "	60°C
5 - "	58°C	10 - "	58°C

No product seen ∴ DNA isolated by Invitrogen kit. Nice band seen ∴ DNA from Qiagen kit but least expected size should be 538 bp. The band present appears to be <400 bp.

Repeat PCR rxn ∴ following modification:

- add 73.7 µl dH_2O
- aliquot 23 µl of master mix into PCR tubes
- add 2 µl template DNA
- change annealing temp for Invitrogen template to 56°, 54°, 52°, 50°C; change annealing temp for Qiagen template to 58°, 56°, 54°, 52°C.

1 - 100 bp ladder		6 - 100 bp ladder	
2 - A1 (Invitrogen)	56°C	7 - A1(Qiagen)	58°C
3 - "	54°C	8 - "	56°C
4 - "	52°C	9 - "	54°C
5 - "	50°C	10 - "	52°C

Now have band in all samples. However, suppressor band present in both samples yet appears to be <500 bp, still smaller than expected.

β_2AR PCR (Small) 3/1/96

Set up master mix for four (4) 25 ul PCR rxns:

2 ul	template DNA (DWm)
1.5 ul	forward primer
1.5 ul	reverse primer
20 ul	5X buffer (buffer N from Invitrogen PCR optimizer kit)
10 ul	dNTPs (2.5mM) (from optimizer kit)
65 ul	dH_2O
0.8 ul	Taq
100 ul	total

- aliquot 25 ul of master mix into 4 PCR rxn tubes
- overlay $\frac{1}{2}$ drop of mineral oil
- PCR cycle

98°C	2 min	
98°C	30 sec	} 30 cycles
56° 54° 52° or 50°	30 sec	} 30 cycles
72°C	30 sec	} 30 cycles
72°C	7 min	

- remove 10 ul aliquot + run on minigel

#1 - 100 bp ladder
#2 - 56°C
#3 - 54°C
#4 - 52°C
#5 - 50°C